

What is claimed is:

1. A method for altering an endonuclease recognition site
5 specificity, comprising:

(a) subjecting a mutagenized endonuclease gene library to a
genetic selection in a population of prokaryotic host cells
expressing one or more non-cognate DNA methyltransferases,
10 wherein the genetic selection selects for viable cells in the
population; and

(b) identifying whether the viable cells express an active
mutated endonuclease with an altered recognition site
15 specificity.

2. A method according to claim 1, wherein the mutagenized
endonuclease gene library is formed by: error prone PCR, chemical
mutagenesis, assembly PCR, DNA shuffling, *in vivo* mutagenesis,
20 cassette mutagenesis, recursive ensemble mutagenesis or
exponential ensemble mutagenesis.

3. A method according to claim 1, wherein the endonuclease
activity is attenuated.
25

4. A method according to claim 3, wherein attenuating the
activity of the endonuclease expressed by the mutagenized
endonuclease gene library is achieved by modifying the mutagenized
endonuclease gene library using modification means selected from:

creating an amber codon within the open reading frame; creating an opal codon within the open reading frame; changing the start codon to GTG or TTG; mutating the RBS sequence or utilizing a T7 expression vector wherein the host cell is T7 RNA polymerase negative.

5

5. A method according to claim 1, wherein step (b) comprises: pooling viable prokaryotic host cells; isolating from the host cells, plasmid DNA encoding mutagenized endonuclease genes from the library; and transforming the plasmid DNA into a population of indicator cells for detecting DNA damage.

10

6. A method according to claim 5, wherein the mutagenized endonuclease genes are subjected to repeated genetic selections in the population of host cells of claim 1 and in the population of indicator cells.

15

7. A method according to claim 6, wherein the genetic selection in the population of indicator cells comprises a first population of indicator cells lacking a non-cognate methylase(s) and a second population of indicator cells expressing the non-cognate methylase(s).

20

8. A method according to claim 1, wherein altered recognition-site specificity comprises: relaxed recognition-site specificity, increased recognition-site specificity or alternate recognition-site specificity.

25

9. A method according to claim 1, further comprising determining a sequence for the recognition site for the endonuclease.

10. A method for altering recognition site specificity of an endonuclease, comprising:

(a) creating a mutagenized endonuclease gene expression plasmid library from a target endonuclease gene and transforming prokaryotic cells with the mutagenized library, wherein the prokaryotic cells express one or more non-cognate methyltransferases;

(b) selecting prokaryotic cells which are viable after transformation and isolating plasmid DNA from the viable cells;

(c) determining whether the isolated plasmid DNA encodes an active endonuclease by transforming the plasmid DNA into DNA-damage indicator cells;

(d) screening the plasmid DNA encoding the active endonuclease for altered specificity; and

(e) optionally repeating steps (a) through (d) to obtain the endonuclease with altered recognition-site specificity.

11. A method according to claim 10, further comprising: determining the altered recognition site for the endonuclease.

12. A method according to claims 1 or 10 wherein the endonuclease is BstYI.

5 13. A method according to claims 1 or 10 wherein the endonuclease is NotI.

14. A method according to claim 12, wherein the recognition site specificity is altered from 5'-RGATCY-3' to 5'-AGATCT-3'.

10 15. A endonuclease having an altered recognition site specificity wherein the specificity is altered according to claim 1 or claim 10.

16. A modified BstYI enzyme, having a preferred recognition site specificity of 5'-AGATCT-3'.

15 17. A method for modifying recognition site specificity of an endonuclease from a parent specificity to a target specificity, comprising:

20 (a) obtaining a sequence for a plurality of mutated endonucleases obtained according to any of the methods of claims 1 or 10 to determine the mutation(s) for each mutated endonuclease; and

25 (b) mutating a gene encoding the endonuclease to produce one or more of the mutations identified in step (a) so as to produce an endonuclease with the target specificity.